DIPHTHERIA—ITS BACTERIOLOGY.

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HISTORICAL.

The history of the discovery of bacteria in this disease is one full of interest. In 1868, Oertel found micrococci present in the false membranes, and in investigations made in 1874 he found the *Bacterium termo* present. He was even successful in producing croup experimentally in animals, and stated his belief to be that these micrococci were the cause of the disease. Nasililof, Hueter and Tommasi also found micrococci present in this disease, and Klebs, in 1871, and Eberth, in 1872, claimed that the diphtheria micrococci were identical with those of sepsis. At this time the theory of the microbic origin of this disease seemed well founded, but it had many enemies, among the most prominent of whom were Senator and Billroth.

In 1877, Drs. Curtis and Satterthwaite were selected by the New York City Board of Health to investigate the cause of this disease, and as the result of their investigations they reached the conclusion that the so-called diphtheria micrococci were not essential in the production of the disease, and Drs. Wood and Formad, who conducted a series of investigations for the National Board of Health, arrived at the same conclusions. It was thus that the question stood when, in 1883, Klebs first called attention to a bacillus which he found upon the false membranes, which he cultivated, and in 1884 Löffler published his investigations upon the same organism. He was able to separate and cultivate it, and, though not at that time able to produce the typical disease in animals by inoculation, he showed that the organism was poisonous.

His observations were confirmed by Fränkel, and Roux was shortly after successful in producing the disease in animals by using cultures of the bacillus. The organism became known as the Klebs-Löffler bacillus, and is now generally recognised as the cause of diphtheria.

Very valuable contributions to our knowledge of this organism have been made by various observers, among the more recent being those of Kolisko and Paltauf, of Vienna; Ortman, of Königsberg; Escherich, in Munich; Bech, Brieger, Fränkel and Behring, of Berlin; Babes, in Bucharest; Klein, of London; Roux and Yersin, of Paris; and Welch, Abbott, Prudden, Park, Councilman and others, of this country.

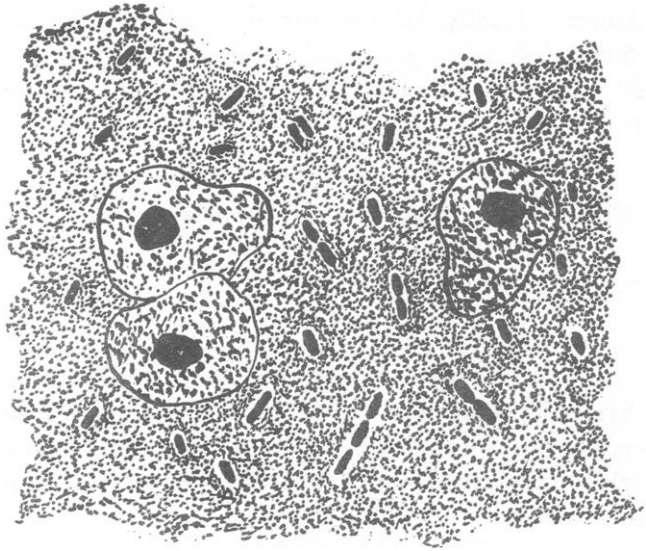
DESCRIPTION.

The Klebs-Löffler bacilli are short rods, straight, or slightly bent, about as long as the tubercle bacillus, but much thicker, and presenting a plump appearance. The ends of the rods are rounded. These bacilli vary greatly in size and appearance under different modes of cultivation, sometimes appearing to be enclosed within a capsule, either as a straight rod or transversely segmented, or oval or elliptical in shape, with swollen ends. Very often, either at the ends or the middle of the bacilli, small round portions, more deeply stained than the remainder of the bacilli are seen, which by some authorities are regarded as spores, but spore formation has not as yet been proved. They are non-motile. In microscopic slides made from portions of false membrane, the bacilli appear in patches and clumps, and also scattered singly over the field examined. The bacilli are found only upon the false membranes and the mucous membranes of the air-passages. They are very resistant, and have been found to be virulent in dried membrane after four weeks. Roux and Yersin found that serum cultures were virulent, under ordinary conditions, after five months, and if protected from heat and light after thirteen months.

Regarding the persistence of the bacilli in the throat during and after recovery from the disease, Park, the inspector

of diphtheria for New York City, reports that of 752 cases examined, 325 were free of bacilli in the throat three days after the disappearance of the membrane, 201 after from five to seven days, 84 after twelve days, 69 after fifteen days, 57 after three weeks, 11 after four weeks, and 5 after five weeks. In one case the bacilli were found after seven weeks, and not only present but virulent.

He also found in healthy persons exposed to the disease, where isolation was not practised, virulent bacilli in 50



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per cent. of the cases examined, forty-five in number, and that, of these, 40 per cent. later developed the disease. Where isolation was carefully attended to, virulent bacilli were found in only 11 per cent. of the cases examined of healthy persons exposed to the disease.

Park and Beebe examined the throats of some 330 persons in New York City, who, as far as known, had not been exposed to diphtheria, and found virulent bacilli present in eight cases.

Park has cultivated virulent bacilli from dried stains on spreads and bed linen, where diphtheritic patients had expec-

torated, and has also shown that the sputum of patients, even after being free from bits of membrane, contains great numbers of the bacilli. Wright and Emerson have obtained virulent cultures from the hair, finger nails, shoes and clothing of nurses to diphtheria patients, and also from chairs, brooms and other objects in the diphtheria ward of the Boston City Hospital, and also in dust collected from the same ward. Williams, of Boston, has found that 50 volume solution of hydrogen dioxide will kill the diphtheria bacillus in five seconds in the laboratory, and recommends it in the treatment, locally, of the disease. A solution of 1-4000 bichloride of mercury is much thought of by Park in the local treatment of the disease. The bacilli perish at a temperature between 45° to 50° C. (112° to 122° F.)

STAINING.

These bacilli do not stain by Gram's method, nor well by the ordinary aniline dye solutions. They stain best by the following method. Make up the following staining solution:

Alcohol sol. methyl-blue	30 c c.
0.01 per cent. sol. caustic potash	100 c. c.

(1 part caustic potash in 10.000 of water.)

Stain the specimens in this for ten minutes, and then place them for fifteen seconds in a ½ per. cent. acetic acid solution. Wash in absolute alcohol and mount in balsam. The bacilli will be stained a dark-blue color.

Crouch, of Denver, in an article recently published,* states that he believes that a direct cover-glass diagnosis of this organism can be generally secured by attention to certain peculiarities in the behavior of this bacillus to certain stains. He found that if a cover-glass prepared as usual, from a blood serum culture, about twenty-four hours old, be treated a few seconds only with methyl-green, in 1 per cent. solution, the following appearances were noticed: "The majority of the bacilli will be stained faintly green and contain at both ends a well-defined round body much more deeply stained, and of a distinctly red color."

* *New York Medical Journal*, October 5, 1895, page 430.

He found the following fluid most serviceable :

1 per cent. sol. methyl-green (freshly prepared)	5 parts.
1 per cent. sol. dahlia (freshly prepared)	1 part.
Distilled water	4 parts.

He states that only a second is required for staining—staining being too intense after a longer time. He further says : "By smearing a piece of the membrane on the cover-glass, drying and flaming in the usual way, and staining (with the above solution) one or two seconds, the diphtheria bacilli, or certain of them, will present the appearances described above. Wherever I have found such forms, even if only two or three, in the direct cover-glass examination, the cultures have developed diphtheria bacilli without one exception, so that I have come to regard this reaction as of the greatest diagnostic importance."

CULTURE.

These bacilli are facultative anerobic, growing best between 60° and 104° F. They grow on gelatine, potato and other media, but the media must be slightly alkaline in reaction. Kolisko and Paltauf found that growth takes place readily upon nutrient bouillon containing sugar. Löffler obtained the best results upon the following media :

Blood serum (cattle)	3 parts.
Beef bouillon	1 part.
Peptone	1 per cent.
Common salt	½ per cent.
Grape sugar	1 per cent.

They also grow especially well upon glycerine agar. Cultures on this media are dull white in color, flat, and present a glistening appearance, with smooth edges and about the size of a millet seed. They develop in from twenty-four to forty-eight hours. Welch and Abbott have found that the bacillus can be cultivated upon potato. Thrust cultures in gelatine are composed of white globular colonies, which develop along the line of puncture.

In some of the large cities of this country the resident board of health makes cultural examinations of suspected cases,

for the convenience of physicians, and the methods employed being of interest, I therefore quote largely from a paper by Park,* the inspector of diphtheria in New York City, regarding the manner of collection and examination of the specimens. The methods described can be carried out well by the general practitioner, where there are no laboratory facilities. Following are quotations from his papers:

"*Technique of Preparing the Serum Tubes and the Swabs.*—The blood is received directly from the slaughtered sheep or calf into large, thoroughly cleansed preserve jars, and covered. These jars are put as soon as possible on the ice. After a few hours the jars should be inspected, and if the clot is found adhering to the sides it should be separated. After twenty-four hours on the ice, the serum is poured off and mixed with one-third its quantity of nutrient bouillon, to which 1 per cent. glucose has been added. This is poured into test tubes, which should be about five inches in length and filled one quarter full. They are placed very obliquely in the serum coagulator, and kept just below 100° C. for one hour on two consecutive days The tubes with the sterile solidified blood serum can then be placed in covered tin boxes and kept for months. The serum prepared in this way is quite opaque, but its value is not lessened for the purpose for which it is intended.

"*The Swab.*—A stiff piece of wire, or, better, a thin steel rod, six inches in length, is roughened at one end by a few blows of a hammer. About this a little absorbent cotton is firmly wound. A number of these are placed in an equal number of glass tubes, the ends of which are plugged with cotton. They are sterilised by dry heat at about 150° C. for one hour, and stored for future use.

"For convenience in carrying the tube containing the blood serum and the tube containing the swab, they are wrapped in a little cotton and placed in a cheap, strong pencil box.

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* Diphtheria and other Pseudo-Membranous Inflammations. *Medical Record*, February 11, 1893.

"*Directions for the Physician to Use in Inoculating the Tubes with the Exudate.*—The patient should first be placed in the best available light, and if a child, properly held. Taking the swab from its tube, the tongue is depressed, and the side of the swab is rubbed firmly against any visible membrane, thus catching little particles in its meshes. Without laying it down, it is inserted the full length of the blood serum tube, and the part of the swab which was previously rubbed against the throat is drawn rather firmly along the full length of the serum surface. It is then re-inserted in its own tube for future use in making control cultures.

"A second culture on a blood serum tube is made from the swab (at the laboratory office). Both serum tubes are placed in an incubator at 37° C. for twelve hours. They are then ready for examination. On inspection the blood serum surface will be seen to be dotted with very numerous, just visible, translucent colonies. At this time no diagnosis can be made by simple inspection. He then takes a clean cover-glass and with a platinum loop makes a sweep over a majority of the colonies, and smears upon the cover-glass, staining with the Löffler methyl-blue, as described under *Staining*. There will then be seen either a large number of characteristic diphtheria bacilli, with a small number of diplo- or streptococci, or a more equal distribution of the true bacilli and the other cocci, or else, where diphtheria is not present, a pure culture of diplococci or streptococci."

Regarding the diagnostic value of this method, he says: "A very extended trial has convinced me that cultures on blood serum, made immediately from the fresh exudate on sterile swabs, can be thoroughly relied upon to show a growth of Löffler bacilli, when these were present and living in the throat at the time of the swabbing, whether visible membrane existed or not."

The method above described can be carried out by the general practitioner, and as a reliable diagnosis of diphtheria is many times of inestimable value in protecting other lives, it should be done where access can not be had to a bacterio-

logical laboratory, and where doubt exists as to the nature of a given case. The general practitioner should be something of a bacteriologist, for many lives often depend upon his diagnosis of just these cases.

EXPERIMENTAL.

Inoculations in animals of cultures are not always followed by success, rabbits, guinea-pigs, chickens and doves being very susceptible, rats and mice not very much so. In the susceptible animals, false membrane is formed in the trachea and sometimes there are constitutional symptoms. Guinea-pigs are very susceptible, and die in a few days after inoculation.

Babes, in a series of experiments, irritated slightly the conjunctivæ of rabbits and then placed upon them pure cultures of the bacillus. The rabbits died in a short time afterward.

Brieger and Fränkel have succeeded in separating a tox-albumin from cultures of the bacilli, which when injected produces albuminuria and paralysis.

IMMUNITY.

Fränkel, Brieger, Kitasato, Behring, Roux and other observers have studied the problem of producing immunity to this disease in animals. Behring and Kitasato have done so by making use of sterilised cultures, by adding iodoform to cultures, and in various other ways, and they found that animals rendered immune are not only safe from the living diphtheria bacilli, but also from the products of their metabolism. Fränkel also obtained immunity by using sterilised cultures.

ANTITOXIN OF DIPHTHERIA.

Of great practical interest is the question of the treatment of this disease by its so-called antitoxin. The principle involved is that the blood serum of an animal rendered immune to the disease, is possessed of curative power when injected into a patient suffering from the disease, by virtue of

an antitoxic substance which has been elaborated. The method of preparing the antitoxin depends upon continuously and progressively injecting a very powerful toxin into an animal ordinarily non-susceptible (the horse is used), and thus the production in this animal's blood of the antitoxin. The antitoxin is in the serum only of the blood.

In 1888, Roux and Yersin discovered and isolated the diphtheria toxine, and to Behring, of Berlin, belongs the credit of the great discovery that the blood of an animal immunised to this disease may be used in treating the disease in the human subject. His observations were published in 1894, and since that time the treatment has been followed out in a great number of cases, both abroad and in this country. In the *Berliner Klinische Wochenschrift*, 1894, No. 36, Behring thus sums up the blood serum therapeutic method:

"1. It is an antitoxic method by which we endeavor to combat those infectious diseases which we know to be of micro-parasitic origin. These include the infectious diseases and certain vegetable and animal poisons (as snake poison) The specific antitoxins, which are the active agents, have until now been found in quantities sufficient to be available for human medication only in the blood of immunised animals.

"2. It is a principle of the blood serum therapy that larger doses are never injurious, but, on the contrary, can be only beneficial.

"3. The blood serum therapy is a specific therapy. Each blood antitoxin is immunising and curative only for one infection.

"4. Under the influence of a specific toxin there is produced a specific antitoxin from the albumin of the living cell. Whilst this is going on there is a disturbance of the regulating mechanism of the general organism. The febrile and other symptoms of a toxic infection are an expression of the effort of the living organism to render the foreign poison innocuous. In animal experiments we can so arrange things that the living organism succeeds. In immunising animals

we render the absorption of even larger quantities of the poison harmless by increasing the antitoxin production.

"5. If we examine the bodily fluids after recovery from an artificial or natural toxic infection, we find not only that the toxin is compensated by the antitoxin, but that there is a surplus of the latter. This surplus is the reason why a larger quanity of the toxin must now be introduced in order to produce intoxication. And this surplus can be employed to help other individuals to overcome the same intoxication. The entire blood serum therapy rests on this fact.

"6. Since these antitoxins are soluble chemical bodies, it is not impossible that they may eventually be produced outside the living body, or even compounded synthetically."

This treatment, as applied to diphtheria, has been elaborated by Aronson, Roux and Yersin, and others, and today stands preëminent as a life-saving measure in this disease. It has received the endorsement of such eminent clinicians as Baginsky, Virchow, Ganghofer, Sonnenberg, Kolisko, Bokai, Moisard, Fischer, Welch, Park, and many others, and should always be tried along with the older methods of combating the disease. In 9,487 cases, tabulated from reports in the *Medical News*, 1894–95, and *Bulletin of Johns Hopkins Hospital*, July–August, 1895, the death-rate is 16.5 per cent. where antitoxin serum was used, while by the old methods alone the death-rate was 47.5 per cent. In the *Deutsche Med. Wochenschrift* (1895, No. 32,) is published a collective investigation of cases treated both with and without the antitoxic serum. In Berlin, of 562 cases treated by serum, 84 died, or 15.1 per cent.; of 282 cases treated without the serum 49 died, or 17.4 per cent. Outside of Berlin, of 5,271 cases treated with the serum 9 per cent. died; and of 4,197 cases treated without the serum 14.4 per cent. died, Totals, 5,833 with serum, 9.6 per cent. died; 4,479 without serum, 14.7 per cent. died. From the foregoing reports, it will be seen that the mortality in this dread disease has been reduced nearly or quite one-half.

A few cases have been reported in which death has been blamed upon the serum, but this is not by any means proven to have been at fault, and considering the immense number of injections which have been made with nothing but favorable results, these cases must be of doubtful nature.

THE PSEUDO-DIPHTHERITIC BACILLUS.

Hoffman, Fränkel, Escherich, Löffler and others have described a bacillus occurring in cases of membranous angina, differing but slightly from the Klebs-Löffler bacillus, some observers even claiming that it cannot be differentiated. They are somewhat shorter and thicker, grow at a temperature of 20–24° C. (68° to 72° F.), forming a mere yellow layer upon agar, and changing the reaction of bouillon less rapidly. They do not grow as well in the presence of oxygen as do the true bacilli. Inoculations into animals sometimes produce local manifestations, but never death.

Hoffman found them frequently in the healthy pharynx; Fränkel, Roux and Yersin think that they are the same bacilli as the Klebs-Löffler, which have in some way lost their virulence. Welch thinks that the name pseudo-diphtheria bacillus should be applied only to bacilli which, though resembling the Klebs-Löffler bacillus, differ from it in being non-virulent, growing more luxuriantly on agar, and the preservation of the alkaline reaction of the bouillon cultures. He holds it to be of a different species than the diphtheria bacillus, and considers it of no diagnostic importance. Paltauf, Sevestre, Baginsky, Martin, Park and Prudden have described cases of membranous disease where the Klebs-Löffler bacillus was absent and only streptococci could be demonstrated. Staphylococci are often found within the false membrane, but they bear no relation to the disease. For clinical purposes, all cases which give bacilli resembling the Klebs-Löffler bacillus in culture and under the microscope should be considered diphtheria.